Program/Abstract # 401
Histamine promotes neuronal differentiation of midbrain neural stem cells
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Histamine (HA) is a biogenic amine with neurotransmitter/neuromodulator properties. In the adult brain, it is involved in the regulation of physiological functions such as sleep, motor activity, as well as thermal and endocrine modulation. During development, HA is one of the first neurotransmitters to appear, and during this period presents higher levels compared with those observed in the adult central nervous system (CNS). Nevertheless, HA function during development has only recently been explored. In this study, we aimed to establish HA effects on ventral midbrain neural stem cells (NSC) from 12 day-old rat embryos. We analyzed the expression of HA receptors (H_1R, H_2R and H_3R) by RT-PCR, and the effect of increasing concentrations of HA on cell proliferation, differentiation and apoptotic cell death. We found that undifferentiated NSC and their differentiated progeny expressed the three types of histaminergic receptors reported in CNS, and that HA modulates their expression differently. Immunocytochemistry studies revealed that 100 nM HA decreased 2.3 times the proportion of tyrosine hydroxylase+ (TH+) cells, and that 10 μM HA significantly increased neuronal differentiation from 20.9 to 36.8% of total cells, as measured by β-tubulin III+ cells, having no effect on the percentage of TH+ cells. No effect of HA on cell proliferation or apoptotic death was observed. These results suggest that micromolar concentrations of HA could be used as a neurogenic factor in midbrain NSC.

doi:10.1016/j.ydbio.2009.05.432

Program/Abstract # 402
Activin A and TGF-β1 participate in the instruction of neural stem cells toward a neuronal phenotype
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Transforming growth factor-β (TGF-β) superfamily members fulfill key functions during development and in tissue homeostasis, through the activation of a complex of type I and type II receptors. During development, several of TGF-β family members and their receptors are expressed in rat cerebral cortex. In the brain, bone morphogenic proteins control neuronal, astroglial and oligodendroglial cell induction. However, little is known about the TGF-β1 and activin A role in cerebrocortical cell differentiation. Studying the effects of activin A and TGF-β1 in neural stem cells (NSC) isolated from rat cerebral cortex of 14 day-old embryos, we found significant increases in the proportion of neurons positive for β-tubulin III after activin A (60%) or TGF-β1 (43%) treatment, relative to untreated cells. Concomitant decreases in the proportion of cells expressing glial fibrillary acidic protein (GFAP) were also observed. In addition, co-treatment with activin A and SB431542 (TGF-β receptor type I inhibitor), decreased the proportion of neurons positive for β-tubulin III (−87.47%), while increased the proportion of immunopositive GFAP cells to 51%. Similar results were obtained when NSC were treated with TGF-β1 and LY364947 (selective inhibitor for Alk5): a decrease in β-tubulin III cells (−65%), and an increase in GFAP cells (33%). In summary, these findings suggest that activin A and TGF-β1 participate in the instruction of NSC toward a neuronal phenotype and that the effect of both cytokines could be mediated by common intracellular pathways, since the effects observed are quite similar.

doi:10.1016/j.ydbio.2009.05.433

Program/Abstract # 403
Molecular regulation of primitive endoderm development
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Primitive endoderm progenitors are first evident within the inner cell mass (ICM) of the early mouse blastocyst, intermixed with the presumptive epiblast population. Two Sox transcription factors, Sox7 and Sox17 are commonly used as endoderm markers. Though null embryos for both Sox7 and Sox17 display no extraembryonic endoderm defects, an essential role for this SoxF sub-family may be masked by their redundant expression. To address the molecular function of Sox7/17 we first used retinoic acid-induced differentiation of F9 mouse embryonic carcinoma cells. These cells transition from epiblast-like cells to primitive endoderm-like cells within 3–4 days of differentiation. Gene expression arrays of F9 differentiation including the analysis of Sox17 knockdowns uncover genes that are potentially downstream of Sox7/17 in primitive endoderm formation. At the same time, Sox17 proteins are also found specifically in the primitive endoderm layer by the late blastocyst stage of mouse embryos and as such, would be an interesting and relevant gene to study with respect to primitive endoderm formation.

doi:10.1016/j.ydbio.2009.05.434

Program/Abstract # 404
Lineage tracing of Hes1+ cells reveals a dynamic contribution of Notch-responsive cells to endodermal organs of the mouse
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Notch signaling regulates cell fate decisions during embryogenesis and in the adult. We are interested in its roles in the vertebrate endoderm, including the pancreas. *Hes1*, a downstream target and effector of Notch, is widely expressed by early pancreatic progenitor cells and apparently downregulated during differentiation. It is unclear if *Hes1*$^+$ cells are multipotent throughout pancreatic organogenesis, or if their differentiation competence changes over time. Similarly, although *Hes1* is known to be expressed in a subset of adult pancreatic duct cells, it is unknown if these cells exhibit progenitor behavior analogous to their embryonic counterparts. Notch signaling through *Hes1* is also thought to play important roles in liver and intestine development, although the dynamics of *Hes1* expression in these tissues is less well-studied. We are using lineage-tracing in the mouse to address these and other issues. We have generated a *Hes1*^{CreERT2} knock-in allele that allows us to mark and trace *Hes1*$^+$ cells in embryos and adults. We find that early *Hes1*$^+$ cells are indeed multipotent in the early embryonic pancreas, but that their potency becomes restricted to exocrine cells as development proceeds. As the pancreatic contribution of *Hes1*$^+$ cells declines, their contribution to the intestinal epithelium and intrahepatic bile ducts increases. We are extending these studies to determine the contribution of *Hes1*$^+$ cells to adult pancreas, liver and intestines, as well as determining whether Notch activation or inhibition regulates the differentiation potential of these cells.

doi:10.1016/j.ydbio.2009.05.435

Program/Abstract # 405
Ngn3 is required for both endocrine islet cell differentiation during embryogenesis and function in adult stages
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Ngn3 is both necessary and sufficient to induce endocrine islet cell differentiation from endodermal progenitor cells during embryogenesis. Because robust *Ngn3* expression has not been detected in hormone-expressing pancreatic islet cells, Ngn3 is utilized as an endocrine progenitor marker and regarded dispensable for the function of differentiated islet cells. Thus, detection of *Ngn3* expression in the adult pancreatic cells was interpreted as evidence of the presence of endocrine progenitors or stem cells. Here we utilized *Ngn3-CreER* knock-in reporter mice and mRNA/protein-based assays to examine *Ngn3* expression in hormone-expressing islet cells. We showed that Ngn3 mRNA and protein are detected in hormone-producing cells at both embryonic and adult stages. Significantly, inactivating Ngn3 in insulin-expressing β cells at embryonic stages or in Pdx1-expressing islet cells in the adults impairs endocrine function, a phenotype that is accompanied by a reduced expression of several Ngn3 target genes that are essential for islet cell differentiation, maturation, and function. These findings demonstrate that Ngn3 is required for not only initiating endocrine cell differentiation, but also islet cell maturation and functional maintenance, and Ngn3 production in the adult pancreatic cells cannot be utilized as an endocrine progenitor marker.

doi:10.1016/j.ydbio.2009.05.436

Program/Abstract # 406
Erythroid development in the absence of hemoglobin
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The mammalian erythrocyte is a highly specialized blood cell that differentiates via an orderly series of committed progenitors in the bone marrow in a process termed as erythropoiesis. In mature red blood cells over 95% of the protein is hemoglobin (Hb) that consists of two alpha and two beta globin polypeptide chains. What happens during erythropoiesis in the absence of hemoglobin? To answer this question, we generated homozygous alpha and beta globin knockout (Null Hb) embryos, adult chimeric mice using novel Null Hb embryonic stem cells (Null Hb ES), and an in vitro ES cell derived erythroid progenitor (ES-EP) culture system. Null Hb embryos died at ~12.5 d in utero. Committed Null Hb erythroid progenitors were present, but did not differentiate beyond the basophilic erythroblast stage. EKLF was tagged by EGFP to track Null Hb ES cells derived from erythroid cells in chimeras. Analysis of adult chimeric bone marrow revealed that Null Hb derived white blood cells developed normally, but the erythroid lineage was again blocked at the basophilic erythroblast stage. In vitro Null Hb ES-EP cultures could support the growth and expansion of Null Hb proerythroblasts; however, upon terminal differentiation Null Hb ES-EP cells undergo apoptosis and cell death. Expression of human myoglobin targeted to the beta globin locus in Null Hb ES cells could rescue erythroid development in the bone marrow of chimeras. These experiments demonstrate that Hb is not necessary for erythroid lineage commitment, is required for terminal erythroid differentiation, and that human myoglobin can rescue erythroid development in the absence of Hb.

doi:10.1016/j.ydbio.2009.05.437

Program/Abstract # 407
Basal cells as stem cells of the mouse trachea and human conducting airways
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The epithelial cells of the respiratory epithelium of mice and humans, constantly exposed to inhaled toxins and pathogens, are maintained over the long term via controlled division of adult tissue stem cells. We have demonstrated that basal cells (BCs) of the mouse trachea give rise to both Clara and ciliated cells by in vivo lineage tracing. Furthermore, we have developed a novel culture system to assay the self-renewal and differentiation of BCs. To identify mechanisms that regulate these behaviors, we have purified BCs by fluorescence activated cell sorting and performed microarray analysis. Using mutant mice and in vitro assays, we are currently testing the hypothesis that genes expressed at high levels in BCs, including transcription factors, signaling molecules, and cytoskeletal components, control their proliferation, differentiation, and motility both at a steady-state and in response to epithelial injury. Finally, we have determined that p63-expressing cells are present even within the smallest conducting airways of humans. Characterization of this stem cell population in mice and humans should enhance our understanding of pathological conditions of the airways including chronic asthma, chronic obstructive pulmonary disease, and cancer.

doi:10.1016/j.ydbio.2009.05.438

Program/Abstract # 408
Bone marrow-derived macrophages fuse with intestinal epithelium in the stem cell niche after injury
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Adult bone marrow-derived cells (BMDCs) can engraft into damaged intestinal epithelium of mice representing a potential avenue for facilitating tissue regeneration. The underlying mechanism for this BMDC engraftment occurs by cell fusion, analogous to cell fusion that occurs during development. We previously identified the intestinal stem cell as the fusion target, but the marrow-derived fusion partner remains unknown. Here we identified the macrophage population as the primary BMDC fusion partner by isolation and transplantation of discrete hematopoietic lineages into recipient mice. Transplantation of isolated macrophages supported robust intestinal epithelial fusion at levels equivalent to whole bone marrow. Additionally, a close examination of the time course for cell fusion reveals that macrophages are among the first cell types recruited to the intestine after injury and surround the stem cell niche. Interestingly, the fusion hybrid cells are not multinucleate, indicating they may be reprogrammed. Indeed expression of macrophage genes was sustained in long-lived fusion hybrids. These studies are the first to illustrate the critical temporal window for visualization of cell fusion after injury. Importantly, understanding the timing and cellular players involved in cell fusion establishes the foundation for further investigations into the molecular mechanism. Establishing the temporal dynamics of fusion and subsequent genetic reprogramming in cell fusion hybrids may provide insight into the physiologic impact of cell fusion in regeneration and susceptibility to disease.

doi:10.1016/j.ydbio.2009.05.439

Program/Abstract # 409
Identifying gene regulatory networks that control adult regeneration in zebrafish
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